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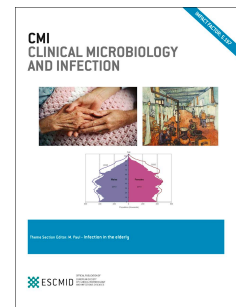
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## **Early Antibody Response in Healthcare Professionals after Two Doses of SARS-CoV-2 mRNA Vaccine (BNT162b2)**

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**Abstract**

**Objectives** Data on the immune response after two doses of BNT162b2 are so far limited. Previously infected individuals were excluded from pivotal clinical trials and the optimal dose regimen in this population has not been clearly studied. The CRO-VAX HCP study aims at investigate the early antibody response in a population of healthcare professionals having received two doses of the BNT162b2 mRNA COVID-19 vaccine.

**Methods:** The CRO-VAX HCP study is a multicenter, prospective, interventional study conducted in several sites in Belgium. The study included 231 healthcare professional volunteers who received the two-dose regimen of the BNT162b2 mRNA COVID-19 vaccine. Of these, 73 were previously infected by SARS-CoV-2 and 158 were uninfected and seronegative. In the first group, blood samples were collected at baseline and after 2, 4, 7, 10, 14, 21, and 28 days. In the second group, samples were obtained at baseline and after 14 and 28 days. Antibodies against the SARS-CoV-2 nucleocapsid and the receptor binding domain of the S1 subunit of the spike protein were measured in all individuals at different time points.

**Results:** In uninfected individuals, 95.5% (95% CI 91.0-98.2%) developed anti-spike antibodies after 14 days and a 24.9-fold rise (95% CI 21.4-28.9%) in antibody titer was observed after the second dose. In previously infected individuals, peak antibody response was reached after 7 days (i.e. 6,347 U/mL) and the second dose did not lead to significantly higher antibody titers (i.e. 8,856 to 11,911 U/mL). Antibody titers were higher in previously infected individuals.

**Conclusions:** This study supports the concept that a single dose of BNT162b2 would be sufficient in previously infected individuals.

**Introduction:**

The efficacy and safety of the two-dose regimen BNT162b2 mRNA COVID-19 vaccine (Pfizer-BioNTech, Mainz, Germany) has been proved and led in late December to its approval by several regulatory authorities [1-3]. Nevertheless, data on the immune response after two doses of BNT162b2 are so far limited [4-7]. Additionally, individuals who had previous clinical or microbiological diagnosis of COVID-19 were excluded from pivotal clinical trials [2, 3, 6], precluding the evaluation of the vaccine response in this particular subpopulation.

**Methods:**

The CRO-VAX HCP study is a multicenter, prospective and interventional study designed to assess the antibody response in a population of healthcare professionals having received two doses of the BNT162b2 mRNA COVID-19 vaccine. Two-hundred and thirty-one volunteers from 3 medical centers in Belgium were enrolled. All participants provided informed consent prior to collection of data and specimen. The study was approved by the ethical committees of the 3 medical centers (approval number: 2020-006149-21). Participants received the first vaccine dose from January 18, 2021, to February 17, 2021. The second dose was administered 21 days after the first one. All volunteers underwent a blood drawn within 2 days before the first vaccine dose. Volunteers were then included in two follow-up protocols in a 1:2 ratio. In the first group, samples were collected at baseline and after 2, 4, 7, 10, 14, 21, and 28 days while in the second group, samples were obtained at baseline and after 14 and 28 days.

Antibodies against the SARS-CoV-2 nucleocapsid (anti-NCP; Elecsys Anti-SARS-CoV-2 NCP qualitative ECLIA, Roche Diagnostics, Machelen, Belgium) and the receptor binding domain of the S1 subunit of the spike protein (anti-S; Elecsys anti-SARS-CoV-2 spike quantitative ECLIA, Roche Diagnostics) were measured at each time point in all serum samples.

Statistical analysis was performed with GraphPad Prism 9.0.1 (GraphPad Software). Antibody titers between groups were tested using a Dunn's multiple comparisons test, with  $P < 0.05$  considered significant.

**Results:**

In our cohort, 73.6% ( $n = 170$ ) were females (mean age = 42.6 years; range, 23-66 years) and 26.4% ( $n = 61$ ) were males (mean age = 42.8 years; range, 23-64 years). Sixty-five persons had a previous positive RT-PCR diagnosis (mean days since RT-PCR = 99; range, 34-337). Among these, 63 persons had symptoms while only 2 were asymptomatic, none requiring hospitalization. Eight additional

participants with positive anti-NCP antibodies at baseline but without evidence of clinical or microbiological diagnosis of COVID-19 in the past were recategorized as previous COVID-19 positive patients (detailed information of the population is presented in **Supplementary Table 1**).

In uninfected, seronegative individuals, the rate of seroconversion after the first dose was 55.6% (95% confidence interval (CI) 41.4-69.1%) and 95.5% (95% CI 91.0-98.2%) at day 10 and 14, respectively (**Figure 1**). Among individuals included in the first group, none had positive anti-S antibodies before day 4 and only one participant seroconverted at day 7 (1.8%; 95% CI 0.1-9.4%). From day 21, all participants had detectable anti-S antibodies (100%; 95% CI 93.3-100%). At day 28 and following the second vaccine dose, a 24.9-fold (95% CI 21.4-28.9) increase was observed compared to day 21.

In individuals with a previous clinical or microbiological diagnosis of COVID-19, no change in anti-S titers was observed up to day 4. Only 5 samples from 3 previously participants with a previous molecular diagnosis of SARS-CoV-2 infection but who were seronegative at inclusion turned seropositive after 4 days. At day 7, a significant 139.9-fold (95% CI 110.8-172.1) increase in anti-S titers was observed. Following the second dose, a 262.4-fold (95% CI 228.1-294.4) increase from baseline was observed. Nevertheless, mean titers at days 14 (i.e. 7,437 U/mL), 21 (i.e. 8,856 U/mL) and 28 (i.e. 11,911 U/mL) were non-significantly different from those at day 7 (6,347 U/mL) ( $P > 0.99$ ). Anti-NCP titers remained unchanged over the 28 days (**Supplementary Figure 1**).

Considering each time point separately, anti-S titers of previously infected individuals were always statistically higher compared to uninfected individuals (**Supplementary Table 2**). At day 7, anti-S titers from previously infected individuals (i.e. 6,347 U/mL) were non-significantly different from titers detected after the second dose of BNT162b2 in previously uninfected individuals (i.e. 1,312 U/mL). From 14 days after the first dose of BNT162b2 anti-S titers of uninfected individuals (from 13.5 to 52.7 U/mL) were similar to anti-S titers of individuals with a previous clinical or microbiological diagnosis of COVID-19 at baseline (45.4 U/mL) ( $P > 0.99$ ). After the second dose, anti-S titers of uninfected individuals (i.e. 1,312 U/mL) were statistically higher compared to baseline levels of previously infected individuals (i.e. 45.4 U/mL;  $P < 0.0001$ ).

## Discussion:

In this study, we report a stronger humoral response in individuals with previous SARS-CoV-2 infection after the first dose of BNT162b2, supporting the concept that this first dose would act as a boost of a previous immunization, as also observed by others [4-7]. This is further supported by the non-significant increase in antibody titers reported after the second dose compared to antibody titers already observed 7 days after the first dose. Evaluation of the pre-vaccinal serological status

could therefore be proposed as a strategy to identify patients who will only require the booster dose [4]. Pan-immunoglobulin assays should be preferred in this context to ensure maximal sensitivity to previous SARS-CoV-2 immunization [8]. Further studies are however needed to determine whether a booster dose in previously infected patients or if a delayed administration of the second dose in uninfected persons could provide sufficient and effective long-term protection.

Our study has some limitations. The findings should be completed by the assessment of the neutralizing capacity of the anti-S antibodies and by investigation of the cellular immune response. Importantly, while the conclusions of this study are of interest to support the concept of a single booster dose strategy in previously infected individuals, the efficacy of this dose regimen should be confirmed in a sufficiently powered study evaluating clinical outcomes.

This study (EudraCT registration number: 2020-006149-21) has a planned follow-up of two years. We would therefore be able to determine the long-term kinetics of the humoral response in both uninfected and previously infected participants.

**Author contributions:** study design: JF, JLB, FM, JMD, MC, JD; sample analysis: JF, JLB, MC; data collection: JF, JLB, MC; data analysis: JF, JLB, FM, JMD, MC, JD; statistical analysis: JF; interpretation: JF, JLB, FM, JMD, MC, JD; writing: JF; and supervision: JMD, JD.

**Conflict of interest Disclosures:** The authors have nothing to disclose.

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**Figure 1:** Evolution of SARS-CoV-2 spike antibodies (U/mL) in individuals with previous SARS-CoV-2 infection (red points) and in seronegative persons without declared history of infection (blue points). Blood samplings before the first vaccine dose were obtained maximum 2 days before. Geometric means with 95% confidence intervals are shown, if applicable. The grey dotted line corresponds to the positivity cut-off (i.e. 0.8 U/mL) of the Elecsys anti-SARS-CoV-2 spike quantitative ECLIA. An automatic dilution of 1/100 at >250 U/mL was performed by the analyzer to extend the measurement domain up to 25,000 U/mL. Forty-two samples were rounded to 25,000 U/mL out of 1,038 (4%). Results < 0.4 U/mL (limit of quantification) were rounded to 0.4. Up to day 4, blood samplings performed one day earlier or later compared to the expected blood times collection were allowed. From day 7, two days were allowed. Individuals with incomplete samplings were not excluded from the analysis.

